

Figure 1

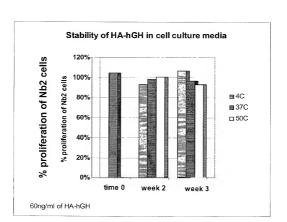


Figure 2

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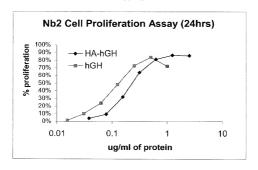


Figure 3A

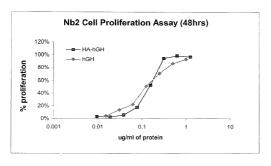


Figure 3B

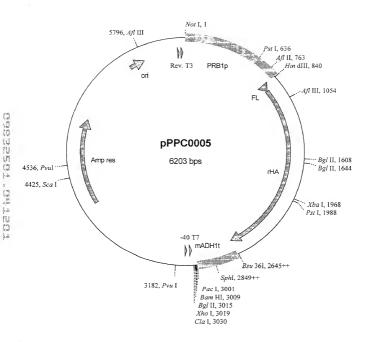


Figure 4

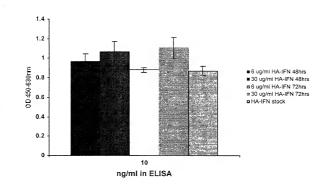


Figure 5

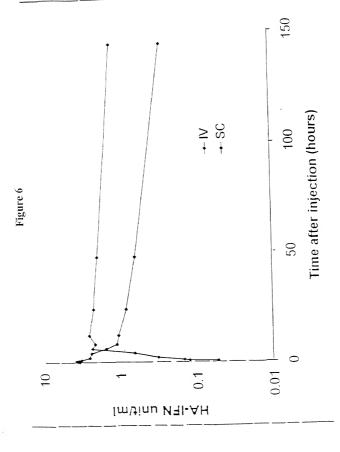
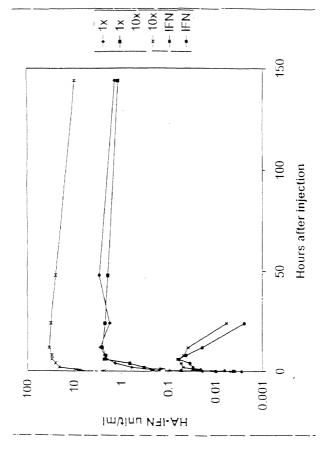


Figure 7



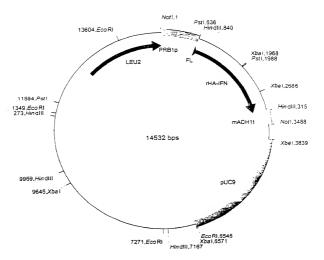


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises

PRB1 promoter, from S. cerevisiae.

Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MFα-1 leader.

HA-IFNa coding sequence with a double stop codon (TAATAA)

ADH1 terminator, from S. cerevisiae. Modified to remove all the coding sequence normaly present in the Hind III/BamHI fragment generally used.

Figure 8

<u>Localisation of 'Loops' based on the HA Crystal Structure</u> <u>which could be used for Mutation/Insertion</u>

1	DAHKSEVAHR FKDLGEENFK HHHHH HHH HHH	ALVLIAFAQY ННННННННН	LQQCPFEDHV HHHHH	KLVNEVTEFA НИННИНИНИ					
	_		TT	III					
	Ι								
51	KTCVADESAE NCDKSLHTLF	HHHHH	<u>кетудема</u> рс	н нннн					
101	CFLQHKDDNP NLPRLVRPEV HHHH H	DVMCTAFHDN HHHHHHHH	EETFLKKYLY HHHHHHHHH	EIARRHPYFY HHHHH					
		TV							
151	APELLFFAKR YKAAFTECCO	AADKAACLLP HHHHH	KLDELRDEGK НННЕННННН	ASSAKQRLKC НИННИНИНИ					
				V					
201	ASLQKFGERA FKAWAVARLS ННННН НН ННННННННН	QRFPKAEFAE HH HHH	VSKLVTDLTK HHHHHHHHHH	VHTECC HGDL HHHHHHH HH					
VI VII									
251	LECADDRADL AKYICENODS			AEVENDEMPA					
251	нинининин нинин	ннннн	нннннн	Н					
301		EAKDVFLGMF HHHHHHH	LYEYARRHPD HHHHHH	YSVVLLLRLA HHHHHHHH					
	VIII								
351	KTYETTLEKC CAAADPHECY	AKVFDEFKPL H HHHHH	VEEPQNLIKQ	NCELFEQUE					
	нинининн нн	н нипип	nnnnnnnnn	THITHHHHA					
				IX					
401	YKFQNALLVR YTKKVPQVST	PTLVEVSRNL	GKVGSKCCKH	PEAKRMPCAE					
	нинининини н	нинининин	HHH	ннннннн					
			XI						
	X DYLSVVLNQL CVLHEKTPVS	DOWNYCOTES		A LEVDETYVPK					
451	HHHHHHHHHH HHHHH		нининин	H					
501	EFNAETFTFH ADICTLSEKE HHH HHE	RQIKKQTALV HHHHMMEHHH	ELVKHKPKAT HHH	HHHHHHHH KEQLKAVMDD					
	XII FAAFVEKCCK ADDKETCFAE	POWNINACO	AAT.CT.						
221		HHHHHHHHHH							
	111111111111111111111111111111111111111								
	Loop	Loop		-222					
	I Val54-Asn61		Glu280-His						
	II Thr76-Asp89 III Ala92-Glul00	ATTT	Ala362-Glu Lys439-Pro	1300					
	IV Gln170-Ala176		Val462-Lys						
	V His247-Glu252	XI	Thr478-Pro						
	TIT C1,,200 C1,,277	YTT	Larg 560 - Th	r566					

Figure 9

XII

Lys560-Thr566

VI Glu266-Glu277

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

IV

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.



ĽV

151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

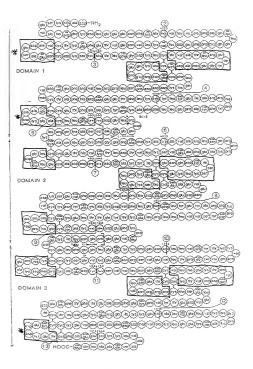


Figure 11

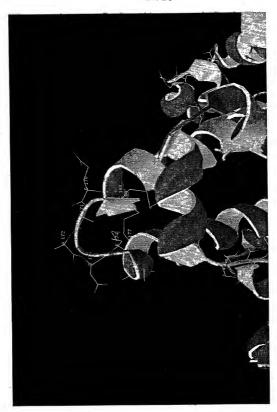


Figure 12: Loop IV Gln170-Ala176 Disulfide bonds shown in yellow

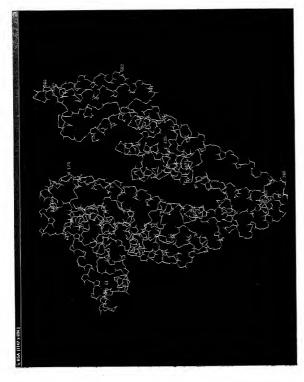
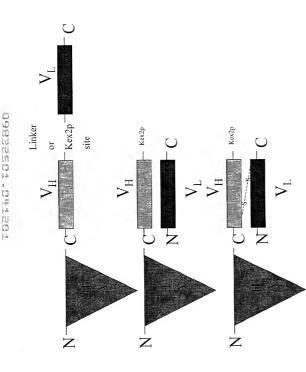


Figure 13: Tertiary Structure of HA



(Example is of a C-terminal fusion to HA) Figure 14: Schematic Diagram of Possible ScFv Fusions

1 GAT GCA CAC ARG AGT GAT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60 1 D A H K S B V A H R F K D L G E E N F K 20

TTG GIG TTG ATT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120 L V L I A F A Q Y L Q Q C P F B D H V 40

121 AAA TTA GTG AAT GAA GTA ACA TTT GCA AAA ACA TOT GTT GCT GAT GAG TCA GCT GAA 180 41 K L V N B V T B P A K T C V A D B S A E 60

181 ART TOT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240 61 N C D K S L H T L F G D K L C T V A T L 80

GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CT GAG AGA AAT GAA 300 B T Y G B M A D C C A K Q B P B R N B 100 241 CGT (81 R I

120 301 TGC TTC TTG CAA CAC AAA GAT GAC CAC AAAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 101 C F L Q H K D D N P N L P R L V R P E V 361 GAT GTG ATG GTT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420 121 D V M C T A F H D N E B T F L K K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC COG GAA CTC CTT TTT TTT GCT AAA AGG 480

Figure 15A

540 180	200	660	720	780	280	300	32(
CCA	TGT	AGC S	AAA. K	CTT	GAA E	GCT.	GCT.
TTG L	AAA K	CTG	ACC	GAC D	TGT	CCT	TAT
CTG L	CTC	CGC	CTT	GCG A	71GC	ATG	AAC
481 TAT AAA GCT GCT TIT ACA GAA TOT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA	541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600 181 K L D B L R D B G K A S S A K Q R L K C 200	GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660 A S L Q K F G B R A F K A W A V A R L S 220	661 CMG AGA TYT CCC AAA GCT GAG TYT GCA GAA GTT TCC AAG TYA GTG ACA GAY CTT ACC AAA 720 221 Q R F P K A B F A B V S K L V T D L T K 240	GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780 V H T E C C H G D L L E C A D D R A D L 260	781 GCC AAG TAT ATC TOT GAA AAT CAG GAT TCG ATC TCC AGF AAA CTG AAG GAA TGC TGT GAA 846 261 A K Y I C B N Q D S I S S K L K B C C B 28	AAA CCT CTG TTG GAA AAA TCC CAC TGC ATF GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900 K P L L B K S H C I A B V B N D B M P A 300	901 GAC TIG CCT TCA TIA GCT GAT GIT GIT GAA AGT AAG GAT GIT TGC AAA AAC TAT GCT 96(301 D L P S L A A D P V B S K D V C K N Y A 320 C
GCC	CAG	GTG V	ACA	GAC	AAG K	GAT D	TGC
GCT	AAA K	GCA	GTG	GAT	CTG	AAT N	GTT V
AAA K	GCC A	TGG	TTA L	GCT A	AAA K	GAA E	GAT D
GAT D	TCT	GCA	AAG K	TGT	AGT	GTG	AAG K
GCT A	TCG	AAA K	TCC	GAA	TCC	GAA	AGT
GCT A	GCT	TTC	GTT	CII	ATC	GCC A	GAA E
CAA.	AAG K	GCT.	GAA E	CTG L	TCG	ATT	GTT
TGC	999	AGA	GCA	GAT	GAT	TGC	TIT
TGT	GAA E	GAA E	TTT	GGA	CAG	CAC	GAT
GAA.	GAT D	GGA G	GAG .	CAT H	AAT N	TCC	GCT A
ACA	CGG	TTT	GCT	TGC	GAA E	AAA K	GCT
TTT	$^{ m CTT}_{ m L}$	AAA K	AAA K	TGC	TGT	GA.A E	TTA
GCT A	GAA E	CAA	CCC P	GAA B	ATC I	TTG L	TCA
GCT	GAT	CTC	TTT	ACG	TAT	CTG L	CCT
AAA K	CTC	AGT	AGA	CAC H	AAG K	CCT	TTG
TAT	AAG K	GCC	CAG	GTC V	GCC	AAA K	GAC
161	541	601	661	721	781	841	301

Figure 15B

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AAG GAT CCT GAT 1020 331 E A K D V F L G M F L Y E Y A R H P D 340

1021 PAC TCT GTC GTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAQ AAG TGC 1080

1081 TOT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140 361 C A A A D P H E C Y A K V P D E P K P L 380

1141 GTG GAA GAG CCT CAC AAT TTA ATC AAA CAA AAC TGT GAG CTT GAA CAG GTG 1200 381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC ANA TITC CAG AAF GCG CTA TITA GTT CGT TRAC ACG AAG AAA GTA CCC CAA GTG TCA ACT 1260 401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCA ACT CTY GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TOT TGT AAA CAT 1320 421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380 441 P B A K R M P C A B D Y L S V V L N Q L 460

1381 TOT GTO TTG CAT GAO AAA ACO CCA GTA AGT GAC AGA GTG TGC ACA GAG TCC 1440 461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500 481 L V N R R P C P S A L B V D B T Y V P K 500

1501 GAG TIT AAT GCT GAA ACA TIC ACC TTC CAT GCA GAT ATA TGC ACA CIT TCT GAG AAO GAG 1560 501 E F N A E T F T F H A D I C T L S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620 521 R Q I K K Q T A L V E L V K H K P K A T 540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680 541 K E Q L K A V M D D P A A P V E K C C K 560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAAA CTT GTT GCT GCA AGT CAA 1740 561 A D D K B T C F A B B G K K L V A A S Q 580

1741 GCT GCC TTA GGC TTA TAA CAI CTA CAT TTA AAA GCA TCT CAG 1782 581 A A L G L * 585

Figure 151